PCR Amplification

Master Mix for **3 reactions**:

10x Extaq Buffer	6.6 µL
dH2O	37.95 µL
2.5mM dNTP	5.28 µL
Forward Primer	1.32 µL
Reverse Primer	1.32 µL
Template	N/A
Polymerase	0.33 µL

1. Be sure everything above is taken from -20°C and is on ice, fully defrosted before use
2. Create master mix in an eppendorf tube-- add the polymerase last and do not spin once polymerase is added
3. Obtain PCR tubes-- label them all! (But make sure you remember in which order you place into the PCR machine because the marker does come off)
4. Pipet 4µL of water into the PCR tubes
5. Touch a colony with the pipet tip (to get biomass, but not a lot) and suck and release multiple times in the 4µL of water
6. Add 16µL of each master mix into each PCR tube and keep on ice
7. Take tubes and place in PCR Machine
8. Select iGEM folder and chose GA_16S for *G.apicola*, then click 'run'
9. After ~2 hours and 15 mins, open machine and take tubes out- place into the -20°C freezer
10. Turn off PCR machine

GA_16S, PCR Cycle for *G.apicola*

1. 10 min at 95°C
2. 30s at 94°C
3. 30s at 53°C
4. 1.5 min at 72°C
5. Cycle 34 times (steps 2-4)
6. 5 min at 72°C
7. infinity at 12°C